VIROLOGY PROTOCOL

TITLE: Inactivation of Rhinovirus in the Presence of Organic Matter on Soft Surfaces

Formula Number: 1178-172

PURPOSE: To determine the virucidal activity of a test substance which has been analyzed to be at or below the Lower Certified Limit (LCL) which is used to disinfect representative fabric surfaces (i.e. polyester and cotton fabrics).

SPONSOR: Reckitt Benckiser, One Philips Parkway, Montvale, New Jersey, 07645

TEST FACILITY: Virology Laboratory, Reckitt Benckiser, One Philips Parkway, Montvale, New Jersey, 07645

PROPOSED EXPERIMENTAL START DATE: May 23, 2012

PROPOSED EXPERIMENTAL TERMINATION DATE: May 31, 2012

TEST SUBSTANCE IDENTIFICATION:

TEST SUBSTANCE FORMULA NO.: 1178-172		
Batch References	Preparation Dates	Expiration Dates
1836-132	January 18, 2012	January 18, 2014
1836-133	January 18, 2012	January 18, 2014

PROJECT NAME: Brace Low Residue (Lysol Disinfectant Spray) EPA Registration Number 777-99

DESCRIPTION/APPLICATION OF THE TEST SUBSTANCE:
The test substance is an aerosol spray disinfectant. A 2-3 second spray will be applied to the inoculated test surfaces from a distance of 6 to 8 inches for a 10-minute contact time.

DOSAGE LEVEL OF TEST SUBSTANCE:

The results of this study will be valid when the level of active ingredient(s) determined during the chemical characterization of the test substance has been analyzed to be at or below the specified LCL range:

Active Name	Acceptable Levels Less Than or Equal To
Ethanol	56.26%
Onyxide 3300	0.090%

TEST SYSTEM JUSTIFICATION:

Regulatory bodies require that a specific virucidal claim on a hard surface disinfectant be supported by efficacy testing involving treatment of the claimed virus by the test substance. Regulatory bodies will accept adequate data developed by a virological technique which simulates to the extent possible in the laboratory, the conditions under which the product is intended for use. For virucides whose use directions identify the test substance as one intended for use upon hard, inanimate, non-porous environmental surfaces, carrier methods, which are modifications of the AOAC Germicidal Spray Products Test or ASTM E1053 methodology, are to be used in the development of the virological data. The ASTM E1053 method has been modified (and accepted, DP Barcode 391679) to test the efficacy of the test substance on soft surfaces using two representative fabrics. The described experimental design meets these requirements.

PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM:

The components of the test system (i.e. virus, host cell, cell culture media, serum) are all identified through various log-in procedures in the virology lab. Viral cultures are given a unique identification number, logged in upon receipt in the lab, and all viral passages are recorded in the Virus Production Log. Host cell cultures are given a unique identification number, logged in upon receipt in the lab, and all cell passages are recorded in the Cell Passage Log. Prepared cell culture media is assigned a unique reagent number and its preparation is documented in the Reagent Preparation Log. Serum lots are assigned a unique lot number which is documented in the Chemical Log.

All tissue culture plates and flasks used in a specific test will also be labelled with the name of the virus, the formula and batch #'s of the test substances, and the experimental start date.

TEST METHODS / REFERENCE STANDARD OPERATING PROCEDURES:

The procedures found in this protocol are taken from the following references. Any deviations found in this protocol from the following referenced methods are acceptable for demonstration of product efficacy.

1. Soft Surface Disinfection Protocol (Approved and Accepted by the EPA, DP Barcode 391679)

2. Cell Culture Media Preparation and Validation. This method is described in detail in Reckitt Benckiser Microbiology / Virology SOP NO. M/V 011.

3. Quality Control and Validation of Serum Lots. This method is described in detail in Reckitt Benckiser Microbiology / Virology SOP NO. M/V 012.

4. Growth and Maintenance of Mammalian Cells. This method is described in detail in Reckitt Benckiser Microbiology / Virology SOP NO. M/V 013.

5. Virus Stock Production and Maintenance. This method is described in detail in Reckitt Benckiser Microbiology / Virology SOP NO. M/V 015.

6. EPA Virucidal Test Method – Liquid and Spray Disinfectants Against Viruses This method is described in detail in Reckitt Benckiser Microbiology / Virology SOP NO. M/V 022.

7. The methodology used in this assay is a modification of the ASTM Standard Method E1053- 11- STM to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces. The exact methodology that will be performed is described in its entirety in this protocol.

8. ASTM Standard Method E1482-04- STM for Neutralization of Virucidal Agents in Virucidal Efficacy Evaluations

9. The EPA Product Performance Test Guidelines OCSPP 810.2200: Disinfectants for Use on Hard Surfaces- Efficacy Data Recommendations.

VIRUCIDAL EFFICACY EXPERIMENTAL DESIGN:

TEST SYSTEM - ORGANISM:

- Human Rhinovirus 39 (ATCC VR-340)
  The 209 strain of Rhinovirus to be used in this study was originally obtained from the American Type Culture Collection (ATCC). Stock virus was prepared by an external laboratory (Mircobac) by collecting the supernatant culture fluid from infected host cells. The cells were disrupted and cell debris removed by centrifugation. The high titer stock virus was aliquoted and stored at approximately -60°C or below until shipment to Reckitt Benckiser. (The viral propagation record will be provided in the raw data.) At the time of receipt of the frozen stock virus in house, it is logged into the Virus Receipt Log and immediately placed in a deep freezer to be stored at approximately -70°C until used in testing.
- At the time of testing, if necessary, the virus is supplemented with fetal bovine serum to provide an organic soil load of at least 5%. The test virus will contain at least a 5% soil load.

HOST CELLS:

MRC-5 cells (Human, lung, fibroblast cells)
Host Received From:
ViroMed Laboratories,
6101 Blue Circle Drive
Minnetonka, MN 55343

- Semi-confluent to confluent cell plates are bought ready to test from ViroMed Laboratories. Upon receipt of the 24 well plates they are logged in and incubated at least overnight to equilibrate/stabilize after shipping and to reach their desired density. Cell cultures are maintained at 36.0 - 38.0°C at 5-7% CO_2 and used at the appropriate density (confluent).

MAINTENANCE MEDIA (TEST MEDIA):
Minimum Essential Medium (EMEM) + 0.1% Gentamicin + 2% Fetal Bovine Serum (FBS)

There are no known contaminants reasonably expected to be present in the media that are known to interfere with the conduct of the study.

VIRAL CYTOPATHOGENIC EFFECT (CPE):
Highly refractile, rounded cells throughout the monolayer or in loose clusters; 7 to 14 days of incubation at 32.0-34.0°C at 5-7%CO_2.

MEDIA, REAGENTS, AND EQUIPMENT:
Materials
Including, but not limited to the following:

1. Multiwell assay plates (e.g. 24 well). Number of assay plates necessary for a test is dependent upon the number of samples being tested, and the number of dilutions being tested.
2. Sterile glass or plastic serological pipettes (e.g. 2.2 mL, 10 mL, 25mL)
3. 60mm or 100mm (diameter) sterile petri plates
4. Indelible marker
5. Sterile test tubes with closures (for dilution blanks)
6. Sterile test tubes containing 2.0 ± 0.5 g 3mm glass beads with closures
7. Test tube racks
8. Conical 50mLtubes
9. Sterile syringe columns with glass wool
10. Forceps
11. Sterile aspirating pipets (optional)
12. Fabric Test Surfaces – Natural (100 % Cotton)
13. Fabric Test Surfaces – Synthetic (Polyester)
14. 3mm Sterile Glass Beads

Media and Reagents

1. Cell culture maintenance medium (Eagles Minimum Essential Medium (EMEM)) supplemented with Gentamicin (optional) and FBS.
2. Fetal Bovine Serum (FBS)
3. Dulbeccos Phosphate-Buffered Saline (DPBS) (optional)
4. 5% Sephadex LH-20 solution (optional)
5. Trypsin reagent solution (optional)
6. 70-99.9% Ethanol (EtOH) or equivalent
7. Sodium Carbonate (Na_2CO_3)
8. 0.05% isoocyylphenoxypolyethoxyethanol (Triton X-100)

Equipment

1. Biosafety Hood
2. Pipet Aid (optional)
3. CO_2 Incubator
4. Refrigerator/Freezer
5. -70 °C (approximately) Ultra Low Freezer
6. Rees Scientific Environmental Monitoring System
7. Inverted Microscope
8. Vortex Mixer
9. Temperature recording device (e.g. Fluke K/J Thermometer)
10. Aspirating Pump (Vacusafe pump (optional))
11. Centrifuge
12. Bunsen Burner
13. Timer

TEST FABRIC PREPARATION

In this assay, 1 inch by 1 inch fabric squares will be used in place of hard, nonporous surfaces to serve as the test surface to demonstrate disinfection on a fabric surface. Two (2) types of fabrics will be tested. Each fabric type will be tested in triplicate. These fabrics are described below.

1. Fabric Test Surfaces – Natural (100 % Cotton) – approximately 100grams/meter^2, desized and bleached. Source: Style 400 - obtained from Testfabrics, Inc., West Pittston, PA.
2. Fabric Test Surfaces – Synthetic (Spun Polyester Type 54(100% Dacron 54)). Source: Style 777 - obtained from Testfabrics, Inc., West Pittston, PA.

Prior to testing, each fabric will be processed in the following manner. Each fabric type will be processed separately. The documentation of this processing will be recorded.

1. In a stainless steel pot, prepare a fabric scouring solution by adding 1.5 grams Sodium Carbonate (Na_2CO_3) and 1.5 grams Triton X-100 to 3 liters of deionized water while heating.
2. Add approximately 100 grams of test fabric per liter of scouring solution.

3. Allow the solution to reach a rolling boil. Boil for ≥ 60 minutes but ≤ 70 minutes.
4. Using gloved hands and/or tongs, remove the fabric from the scouring solution.
5. Rinse thoroughly with deionized water until all traces (foaming) of the wetting agents are visibly noted to be gone. This can be achieved by running the deionized water to re-fill the pot. To aid in removing the scouring solution, the fabric can be rung out occasionally with gloved hands.

Note: Only gloved hands should be used when handing the fabric from this point forward. This will avoid adding unwanted body oils to the fabric.

6. Allow the fabric to air dry completely by hanging or draping at least overnight.
7. Using scissors and a ruler, cut 1 inch x 1 inch test squares from the dried fabric.
8. Place each test square into separate glass petri dishes.
9. Steam sterilize.
10. Cool and store the fabric test squares at ambient temperatures for ≤30 days.

DESCRIPTION OF EXPERIMENTAL DESIGN:

Number of Dilutions and Cultures for Virucidal Efficacy Study (Per Fabric Type)			
Test or Control Group	**Dilutions Assayed**	**Cultures Per Dilution**	**Total Cultures**
Host Control	N/A	≥ 4	At least 4
Virus Suspension Titer Determination Control	-2, -3, -4, -5, -6, -7, -8	4	28
Dried Virus Control (For Each Fabric Type)	-2, -3, -4, -5, -6, -7	4	24
Sample Batch #1 + Virus (Test in Triplicate for Each Fabric Type)	-1, -2, -3, -4, -5	4	20
Sample Batch #2 + Virus (Test in Triplicate for Each Fabric Type)	-1, -2, -3, -4, -5	4	20
Cytotoxicity Control (One Batch for Each Fabric Type)	-1, -2, -3	4	12
Neutralization Effectiveness Control (One Batch for Each Fabric Type) (Multiple dilutions can be assayed.)	-1, -2, -3	4	12
Neutralization Titer Confirmation	-1 ,-2, -3, -4	4	16

HOST CELL PREPARATION

Multiwell plates (e.g. 24 well assay plates) containing the appropriate host cell line are received from a commercial source (Viromed), logged in and incubated. Prior to testing they are checked for confluency, contamination, etc. Either the day before test, or on the day of the virucidal assay, growth media is aseptically removed from each well containing host cells. The cells can then be washed (i.e. 1 – 2 times) by adding 1-2 mL of wash solution (e.g. DPBS, EBSS or MEM without FBS) to each well, rocking the plate for 10-15 seconds, and removing the wash solution prior to refeeding. This step is optional and can be performed if excessive cell debris is observed. If performed, the wash step will be performed immediately prior to re-feeding the plates and will be documented in the raw data and final report.

FABRIC CARRIER INOCULATION AND DRYING

The virus stock is removed from the freezer (target ≤ - 70 °C) and thawed. A 0.2 mL aliquot of the virus stock is spread onto five areas of the fabric carrier.

1. Inoculate 0.2mL of the test culture onto each sterile carrier using an appropriate micropippette.
2. The inoculum will be applied to five areas of the fabric carrier (the total inoculum of the five areas being 0.2mL). Each corner and the direct center are the target areas. See diagram below:

3. Test surfaces are allowed to dry at ambient temperature (24 ± 5°C) in a laminar flow hood for up to 30 minutes until visibly dry. Alternately, viruses may be dried in a controlled temperature and humidity chamber. Actual drying conditions (temperature and humidity) will be documented in the final report.
4. Aseptically transfer a dried inoculated surface to a sterile petri dish for treatment.

TREATMENT/NEUTRALIZATION OF THE TEST SYSTEM (INOCULATED FABRIC CARRIER) WITH THE TEST SUBSTANCE

Test substance samples are used as per the sponsor's instructions ensuring that the entire test surface area is covered. Note: Only one side (the inoculated surface) is treated with the test substance. For each batch tested, the test substance is applied directly to the dried virus and allowed to sit for the 10 minute contact time at room temperature.

At the contact time, 2.0mL of an appropriate neutralizer will be added to the test surface and the plate will immediately be swirled to mix. Using sterile forceps, the inoculated/treated/neutralized test surface is transferred to a tube containing 2.0 ±0.5g of glass beads. The liquid remaining in the dish after neutralization will be recovered and added to the tube with the test surface. Immediately, vortex the tube for 10-15 seconds. Pass the liquid through Sephadex columns using a syringe plunger, to reduce/eliminate

cytotoxicity. The collected sample will be considered the 10^{-1} dilution of the virus/test substance/neutralizer mixture. Serial tenfold dilutions are then carried out in cell culture media or appropriate neutralizer (e.g. 0.5 mL of a virus dilution is added to 4.5 mL of cell culture media).

Sephadex Gel Filtration to Remove Cytotoxic Effects

- Sephadex columns will be prepared and used in this assay. Sephadex is a filtration medium which, binds to and removes components of the test substance that cause cytotoxicity and aids neutralization of the test substance. Assay controls (Dried Virus Control, Cytotoxicity/Neutralization Effectiveness Controls) must also be passed through Sephadex columns.
- At least one day prior to start of the assay, prepare a 5% Sephadex slurry. Sterilize and store at least overnight at 2-8°C. To sterilize, autoclave the slurry for 20-25 minutes at 121°C/15 PSI, and allow it cool to ambient temperature prior to use.
- Sephadex columns are prepared on the day of the virucidal assay procedure, but the assembly without Sephadex slurry may be prepared earlier and stored until needed. Prepare column by inserting a small wad of glass wool into a syringe barrel to cover the opening in the syringe tip. Place syringe barrel into a sterilized bottle or tube. Autoclave assembly and store at room temperature until needed. On the day of assay, pipet Sephadex slurry into the barrel and allow to drain and settle. Continue to add slurry until the settled column height reaches 8-10 ml in a 10ml barrel, or 4-5 ml in a 5ml barrel. Prior to neutralization, place full columns into a disposable centrifuge tube and cover the top of the tube/barrel assembly. Centrifuge at 550-650 x g in a centrifuge with a swinging bucket rotor for 3-4 minutes to remove the void volume in the column. The height of the column will pack down during this procedure.
- Discard the void volume.
- Neutralized test product/virus mixture, test product/medium mixture or virus/medium mixture will be passed through the Sephadex column utilizing a syringe plunger. The collected mixture (10^{-1} dilution), which was passed through the Sephadex column, will be serially diluted and plated as described.

HOST CELL PLATING AND INCUBATION

A 0.1 mL aliquot of each dilution is then aseptically pipetted into each of four wells of host cells, which were previously aspirated of media, washed (optional) and re-fed using approximately 1-2mLs of the appropriate media. The assay plates are placed under the appropriate incubation conditions, and the cells are observed for toxicity or characteristic viral CPE after the designated time period.

After $\geq$ 22 hours incubation, the plates will be observed for cytotoxicity and any dilutions exhibiting toxicity or potential toxicity will be aspirated and re-fed with the appropriate maintenance media. If a media change out is performed on test plates, it must also be performed on the same dilutions for all controls, except neutralization validation plating and viral titer control. This will be documented in the raw data.

Documentation of the various steps in the procedure as well as results will be recorded in the appropriate data sheet.

CONTROLS

Viral Suspension Titer Determination

The viral suspension will be diluted and plated to determine the initial viral titer of the test virus. A 0.1 mL aliquot of each dilution is aseptically pipetted into each of four wells of host cells, which were previously aspirated of media, washed (optional) and re-fed using approximately 1-2mLs of the appropriate media. The assay plates are placed under the appropriate incubation conditions, and the cells are observed for toxicity or characteristic viral CPE after the designated time period.

Dried Virus Control: (DVC):

A 0.2mL aliquot of the virus inoculum will be inoculated onto the five areas of the fabric test surface and allowed to dry for up to 30 minutes. The actual drying conditions (temperature and humidity) will be recorded and reported in the final report. The DVC will be performed for each fabric test surface type using 2.0mL of media or neutralizer in place of the test substance. At the contact time, 2.0mL of an appropriate neutralizer will be added to the test surface and the plate will immediately be swirled to mix. Using sterile forceps, the inoculated/treated/neutralized test surface is transferred to a tube containing 2.0 ±0.5g of glass beads. The liquid remaining in the dish after neutralization will be recovered and added to the tube with the test surface. Immediately, vortex the tube for 10-15 seconds. Pass the liquid through Sephadex columns using a syringe plunger to reduce cytotoxicity. The collected sample is considered the 10^{-1} dilution of the virus/media/neutralizer mixture.

Serial tenfold dilutions are then carried out in cell culture media or appropriate neutralizer (e.g. 0.5 mL of a virus dilution is added to 4.5 mL of cell culture media). A 0.1 mL aliquot of each dilution is then aseptically pipetted into each of four wells of host cells, which were previously aspirated of media, washed (optional) and re-fed using approximately 1-2mLs of the appropriate media. The assay plates are placed under the appropriate incubation conditions, and the cells are observed for toxicity or characteristic viral CPE after the designated time period.

This control will determine the relative loss in virus infectivity resulting from drying, neutralization and Sephadex neutralization alone.

The results from this control will be compared with the test results to confirm recovery of at least four $\log_{10}$ of infectious virus, for each fabric type, following drying and neutralization. This titer will be compared with the titers of the test results for that particular fabric type to reach the acceptable test criteria.

Cytotoxicity and Neutralization Effectiveness Controls
The cytotoxicity control will be performed for each fabric type to determine the level of cytotoxicity present (TCD_{50}).

The Neutralization Effectiveness Control will determine if residual active ingredient is present after neutralization.

Summary: One lot, one repetition (for each fabric type) of the test substance will be used for the cytotoxicity and neutralizer effectiveness controls. This control will be processed exactly as the test procedure but instead of viral inoculum, appropriate media is added to the surface and dried as described previously. Post test, recovery and Sephadex neutralization, the sample will first be diluted and plated for the cytotoxicity control.

Perform the previous procedure, under Dried Virus Control, utilizing 0.2 mL of cell culture medium as the inoculum, in place of the virus suspension during the inoculation procedure and dry for up to 30 minutes. Treat the surface as performed in the test. At the contact time, 2.0mL of an appropriate neutralizer will be added to the test surface and the plate will immediately be swirled to mix. Using sterile forceps, the media inoculated/treated/neutralized test surface is transferred to a tube containing 2.0 ±0.5g of glass beads. The remaining liquid volume is recovered and added back to the tube containing the test surface and glass beads. Immediately, vortex the tube for 10-15 seconds. Pass the liquid through Sephadex columns using a syringe plunger to reduce cytotoxicity. The collected sample is considered the 10^{-1} dilution of the mixture.

Serial tenfold dilutions are then carried out in cell culture media or appropriate neutralizer (e.g. 0.5 mL of a virus dilution is added to 4.5 mL of cell culture media). A 0.1 mL aliquot of each dilution (of the stock virus) is then aseptically pipetted into each of eight wells of host cells, which were previously aspirated of media, washed (optional) and re-fed using approximately 1-2mLs of the appropriate media. Four wells of each dilution will be used for the Cytotoxicity Control and four wells of each dilution will be used for the Neutralization Effectiveness Control. (Additional host cell wells can be inoculated to accommodate multiple dilutions for the Neutralization Effectiveness Control.) The assay plates are placed under the appropriate incubation conditions and the cells are observed for toxicity or characteristic viral CPE after the designated time period.

Each plated dilution of the neutralized test substance (and Sephadex filtered) will be challenged with a 0.1mL aliquot of low titer virus (which delivers 1-1000 viral particles) to determine the dilution(s) at which virucidal activity, if any is retained. A 0.1mL aliquot of the diluted virus will be added to the four wells of host cells which were previously inoculated with 0.1mL of the neutralized test samples. Those dilutions which are toxic to

the cells and/or do not exhibit virus replication are not included in the $\log_{10}$ reduction calculations of the germicidal activity.

Neutralization Verification Control

The diluted virus used for the Neutralization Effectiveness Control will be plated into host cells as previously described to confirm a low level of viral inoculum (1-1000 viral particles). Each viral dilution used for Neutralization Effectiveness Control will be confirmed.

Host Cells Viability Control

At least four wells will be inoculated with an appropriate media during the incubation phase of the study.

This control will demonstrate that cells remain viable throughout the course of the assay period. In addition, it will confirm the sterility of the test media and fetal bovine serum.

CALCULATIONS:

Viral and cytotoxicity titers will be expressed as $-\log_{10}$ of the 50 percent titration endpoint for infectivity ($TCID_{50}$) or cytotoxicity (TCD_{50}), respectively, as calculated by the method of Spearman Karber.

- Log of 1st dilution inoculated- [((sum of % mortality at each dilution/100) -0.5) x (logarithm of dilution)]

Calculation of log reduction (for each fabric type):

Dried Virus Control $TCID_{50}$ – Average Test Substance $TCID_{50}$ = Log Reduction*
* Note: EPA requires complete inactivation of the test virus.

ASSAY EVALUATION REQUIREMENTS:

The success criteria for soft surfaces will be identical to the EPA virucidal requirements for hard surfaces. The EPA Product Performance Test Guidelines OCSPP 810.2200: Disinfectants for Use on Hard Surfaces- Efficacy Data Recommendations states that the virucidal assay must provide the following information:

1. The virus recovery (titer) must be recovered from a minimum of four determinations per each dilution in the assay system (e.g. cell culture system).
2. Cytotoxicity controls. The effect of the disinfectant on the viral assay system for a minimum of four determinations per each dilution.
3. The activity of the disinfectant against the test virus from a minimum of four determinations per dilution in the assay system be determined.
4. Neutralization Controls. Neutralization controls will be performed from a minimum of four determinations per each dilution in the assay system.

5. The viral preparation method, recovery, and neutralization will be documented in th raw data, including any special methods which are used to increase the virus titer an to detoxify the residual disinfectant.
6. The $TCID_{50}$ values calculated for each assay.
7. The test results shall be reported as the reduction of the virus titer by the activity of the disinfectant ($TCID_{50}$ of the virus control less the $TCID_{50}$ of the test substance assay (test system)), expressed as log_{10}, and calculated by a statistical method (e.g. Spearman-Karber).
8. The product must demonstrate complete inactivation of the virus at all dilutions to b considered efficacious. If cytotoxicity is observed/present in the assay system, at lea a 3 log_{10} reduction in the viral titer must be demonstrated beyond the cytotoxic level The calculated viral titer must be reported with the test results.

Note: At least a 5% soil load of fetal bovine serum will be added to the viral inoculum t simulate an organic soil load (if necessary).

STUDY ACCEPTANCE CRITERIA:

A valid test requires that:

1. The product demonstrates complete inactivation of the virus at all dilutions.
2. When cytotoxicity is evident, a ≥ 3 log_{10} reduction in the viral titer must be demonstrated beyond the cytotoxic level.
3. The virus titer recovered from the dried control must be $\geq 10^4$.
4. The neutralization effectiveness control must demonstrate adequate neutralization o the test substance using low number (1-1000) of viral particles.
5. Host cell controls must be free from contamination and remain viable throughout th length of the assay.

RECORDS TO BE MAINTAINED:

All records that would be required to reconstruct the study and demonstrate adherence to protocol will be maintained. Raw data is entered onto the study raw data sheet, appropriate laboratory notebooks, log sheets, and log books. Supporting records and documents that will be maintained include, but are not limited to, chain of product custody, virus records, media, chemical and reagent preparation and component records, and equipment maintenance and calibration.

SIGNATURE PAGE:

PROTOCOL APPROVAL SIGNATURE:

STUDY SPONSOR: Reckitt Benckiser

SPONSOR REPRESENTATIVE: Ivette Cabello-Straub DATE: May 21, 2012

Ivette Cabello-Straub
SSG Manager

PROTOCOL SIGNATURE:

STUDY DIRECTOR: Kelly Whitehead DATE: May 21, 2012

Kelly Whitehead
Research Associate

MASTER SCHEDULE NUMBER: 2012-0046

Protocol Deviation #1

Master Schedule Study Number: 2012-0046

Formula Number: 1178-172

Protocol Deviations: CO_2 Ranges

1) The protocol stated that the test materials be incubated at 5-7% CO_2.

For the assay, 5-24-2012 to 5-31-2012, the reported CO_2 range was 1.699-7.459%. It is common to have variable CO_2 levels since opening and closing of the incubator door will quickly alter the CO_2 levels. The levels rebound quickly as observed in the Rees readings. The host controls were healthy cells when observed at the end of the assay. Based on the cell controls, it can be concluded that this deviation had no effect on the study or the results reported.

Kelly Whitehead 6/14/12

Study Director- Kelly Whitehead

Ivette Cabello-Straub

Study Sponsor- Ivette Cabello-Straub

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CERTIFICATE OF ANALYSIS
Reckitt Benckiser
Analytical Laboratory
One Philips Parkway, Montvale, NJ 07645

The following batches were analyzed at or below the Lower Certified Limit to be used for Microbiology Testing.

EPA Registration Number	**777-99**
Formula	**1178-172**
Master Schedule Number	**2012-0003**
Fabrication Date	**January 18, 2012**
Dates of Analysis	**February 2, 2012 and April 4, 2012**
Test Method	**GLP-14782E2B-11**
Active Ingredients	**Ethanol and Onyxide 3300**
Acceptance Criteria	**56.26 % Ethanol and 0.090% Onyxide 3300**

Batches	Results at Initial	Results at 60 Days storage at 25C
1836-132	54.88% Ethanol 0.090% Onyxide 3300	55.17% Ethanol 0.087% Onyxide 3300
1836-133	55.15% Ethanol 0.089% Onyxide 3300	54.87% Ethanol 0.088% Onyxide 3300

Prepared By: _____ **Date:** 07/18/2016
Mark Miller
Senior Research Associate Analytical Laboratory

Reviewed by: _____ **Date:** July 18th 2016.
James Clayton
R&D Manager Analytical Laboratory